Molecular Phylogenetics and Evolution 52 (2009) 432–447

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Sandokanid phylogeny based on eight molecular markers—The evolution of a southeast Asian endemic family of Laniatores (Arachnida, Opiliones)

Prashant Sharma *, Gonzalo Giribet

Department of Organismic and Evolutionary Biology & Museum of Comparative Zoology, MCZ Laboratories 507, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA

ARTICLE INFO

Article history:
Received 22 November 2008
Revised 3 March 2009
Accepted 7 March 2009
Available online 24 March 2009

Keywords:
Sandokanidae
Histone H4
Oncopodidae
Indonesia
Thailand
Malaysia
Philippines
Sundaland

ABSTRACT

Little is known about the familial and generic level phylogeny of Laniatores, the most diverse suborder of Opiliones. We investigated the internal phylogeny of the family Sandokanidae (formerly Oncopodidae), the putative sister group of the other families of the highly diverse infraorder Grassatores (Opiliones: Laniatores), on the basis of sequence data from eight molecular loci: 18S rRNA, 28S rRNA, 12S rRNA, 16S rRNA, cytochrome *c* oxidase subunit I (COI), histones H3, H4, and U2 snRNA. Exemplars of all recognized sandokanid genera, as well as a putative new genus from Thailand, were included. Data analyses were based on a direct optimization approach using parsimony, as well as maximum likelihood and Bayesian approaches on static alignments. The results obtained include the monophyly of Sandokanidae and its stability under a variety of parameter sets and methods. The internal phylogeny is relatively robust to parameter choice and demonstrates the monophyly of nearly all described genera, corroborating previous morphological observations. However, conflict among data sets exists with respect to the monophyly of the largest genus *Gnomulus*. Morphological character evolution, particularly of characters used to define genera, such as tarsal count and male genitalia, is reexamined and the performance of the eight molecular markers in phylogenetic estimation is evaluated.

Published by Elsevier Inc.

1. Introduction

While the subordinal relationships of Opiliones (Chelicerata, Arachnida) have received considerable attention (Martens, 1976, 1980, 1986; Martens et al., 1981; Schultz, 1998; Giribet et al., 1999, 2002; Shultz and Regier, 2001), phylogenetic studies within the suborders are few in number, with the notable exception of Cyphophthalmi, which have featured prominently in cladistic and biogeographical analyses (e.g., Giribet and Boyer, 2002; Boyer and Giribet, 2007; Boyer et al., 2007b; Clouse and Giribet, 2007; Sharma and Giribet, in press). Of the four suborders, the largely southern Hemisphere Laniatores encompasses almost two-thirds (over 4000 described species) of described opilionid diversity, but has received far less than commensurate phylogenetic study, with a few exceptions of analyses often restricted to species groups or related genera mostly from South America (e.g., Kury, 1993; Pinto-da-Rocha, 1997; Pinto-da-Rocha and Kury, 2003; Pérez González, 2006).

Subsequent to numerous changes in systematics proposed by many authors over 170 years (reviewed by Kury, 2007), Laniatores is presently divided into two tenuous infraorders, the Insidiatores (Loman, 1900) and the Grassatores (*sensu* Kury, 2003). The former is likely a paraphyletic entity, while the latter harbors most of the order's striking exemplars of morphological, behavioral and ecological diversity. Approximately half of all known Opiliones species (and 22 of the 54 families) are within Grassatores (Kury, 2007). The asymmetrical diversity exhibited by Grassatores with respect to Opiliones recalls that of Coleoptera with respect to Hexapoda.

One particularly exceptional family of Grassatores is Sandokanidae (Thorell, 1876) (formerly Oncopodidae [see Özdikmen and Kury, 2007]), the putative sister taxon to the remaining families within the infraorder (reviewed by Schwendinger, 2007a). Comprising 70 species, Sandokanidae is a comparatively small family, but of tremendous significance, due to the peculiarities of their morphology. Firstly, sandokanids are distinguished from all other Laniatores by a *scutum completum* (fusion of the carapace and opisthosomal tergites I–VIII into a single plate) (Fig. 1), which they share with the most basal lineage of Opiliones, the Cyphophthalmi, and some Troguloidea (Opiliones: Dyspnoi) (Martens and Schwendinger, 1998). By contrast, other Laniatores possess a *scutum magnum* (fusion of the carapace and opisthosomal tergites I–V) (reviewed by Schultz and Pinto-da-Rocha, 2007). Secondly, Sandokanidae are characterized by numerical reductions of the tarsomeres, i.e., articles of the walking leg tarsi. This condition is again similarly found in Cyphophthalmi, wherein males of some species have up to two tarsal articles in leg IV, and Trogulidae, which may have up to four[1] (Martens and Schwendinger, 1998). Tarsomere

* Corresponding author.
E-mail address: psharma@fas.harvard.edu (P. Sharma).

[1] This distinction may be superficial, as Cyphophthalmi are unguligrade (standing on the tarsal edge and the claw), whereas all other Opiliones are plantigrade (standing on the ventral surface of the tarsus only) (Martens and Schwendinger, 1998).

1055-7903/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.ympev.2009.03.013

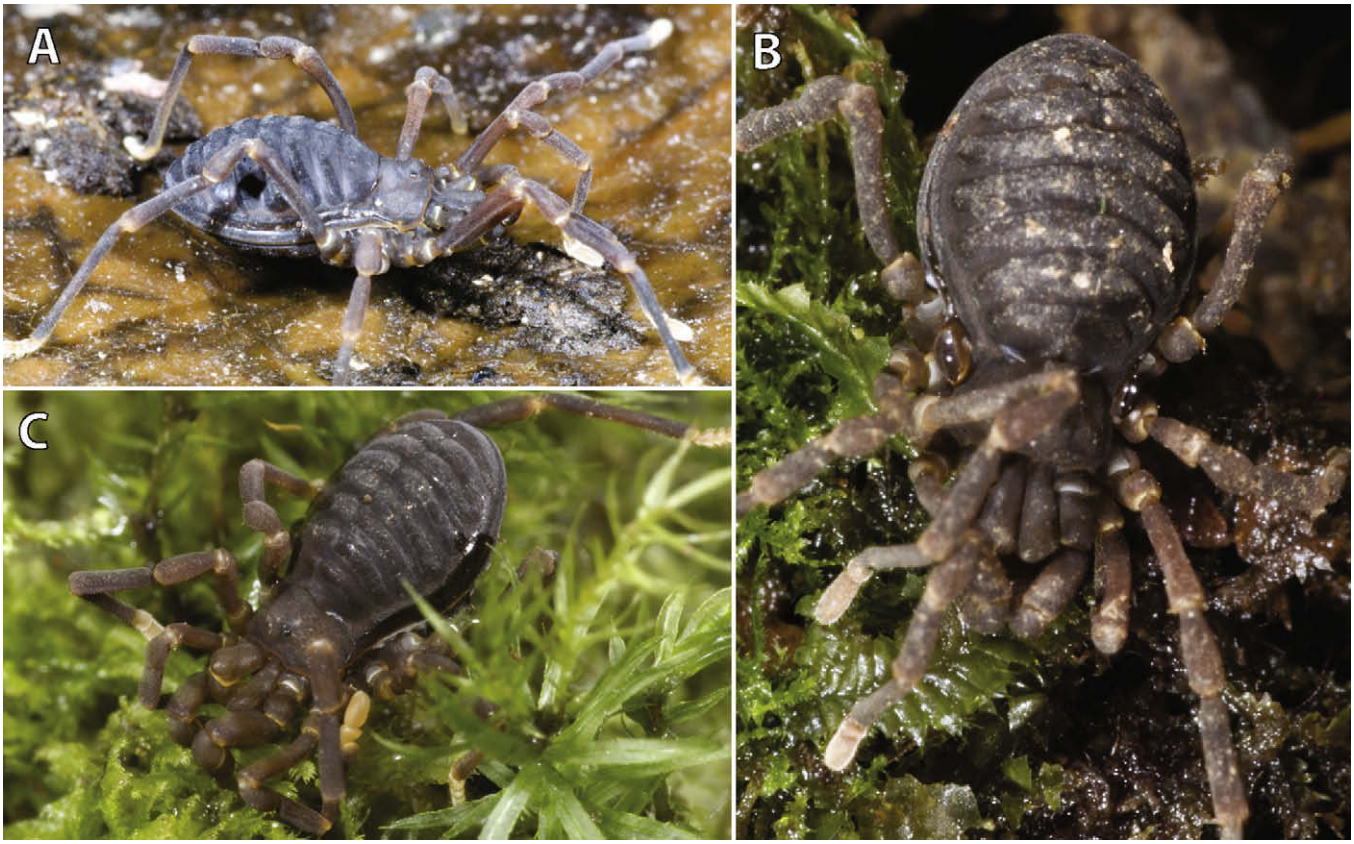

Fig. 1. (A) *Gnomulus latoperculum* from Gunung Tongara, North Sulawesi (Indonesia). (B) *Gnomulus latoperculum* from Gunung Ambang, near Modoinding, North Sulawesi (Indonesia); droplets secreted by repugnatorial glands visible above coxae of walking leg III. (C) *Gnomulus latoperculum* from Gunung Ambang, near Modoinding, North Sulawesi (Indonesia); repugnatorial gland secretion visible spreading along lateral margin of opisthosomal tergites.

number generally correlates with ecology; arboricolous Laniatores (e.g., members of Gonyleptidae) tend to have longer legs with many tarsomeres. Ground-dwelling counterparts (e.g., Cyphopthalmi, Trogulidae and Sandokanidae) typically have shorter legs and reduced tarsomere counts (Curtis and Machado, 2007). An adaptive hypothesis of tarsomere number has yet to be rigorously tested.

Martens and Schwendinger (1998) considered the large scutum and low tarsomere number to be apomorphic reversals in Trogulidae, but assumed they were plesiomorphic characters in Sandokanidae. This may have been due to the lack of a family-level phylogeny of Laniatores and the peculiar morphology of Sandokanidae, which has no clearly identifiable sister group on the basis of morphology alone. Nevertheless, genitalic morphology and cuticular appendages of the carapace were defined as unambiguous apomorphies for the family (Martens and Schwendinger, 1998). Tarsomere number, not greatly variable among the species of Sandokanidae (Table 1), continues to be used to delineate generic boundaries in conjunction with genitalic morphology. The exception, *Pelitnus* Thorell, 1891, was distinguished from *Gnomulus* only by a protuberance on the ocularium (Schwendinger, 1992). *Pelitnus* was later synonymized with *Gnomulus* (Martens and Schwendinger, 1998).

Furthermore, the distribution of Sandokanidae places them at the forefront of a biogeographical quandary, i.e., the origin of the Malay Archipelago (Hall, 2002). Sandokanidae are known from northeastern India, Nepal, China, Thailand, Vietnam, the Malay Archipelago, Sundaland and the Philippines. This distribution, combined with the categorization of Sundaland as a biodiversity hotspot for conservation priority (Myers et al., 2000), suggests that Sandokanidae may prove informative in studies of the Malay Archipelago's biogeographical history. The relevance of sandokanid biogeography is augmented by ongoing study of the family Stylocellidae (Opiliones, Cyphophthalmi), which has a nearly identical distribution (Boyer et al., 2007b; Clouse and Giribet, 2007). Moreover, both Stylocellidae and Sandokanidae share important ecological characteristics, such as cryptic lifestyle, leaf-litter habitats, and limited dispersal ability. The potential for study of cladistic biogeography with complementary data sets from two independent opilionid lineages is enormous.

To abet forthcoming studies of this cryptic family of Laniatores, we investigated the generic level relationships of Sandok-

Table 1
Described genera of Sandokanidae and significant morphological characteristics. Tarsal formula is defined as the number of tarsal articles on the walking legs of adults, from leg I to leg IV. Glans direction refers to the orientation of the glans in the penes of adult males.

Genus	Tarsal formula	Glans direction	Described species
Sandokan (Thorell, 1876)	1:1:1:1	Proximad	9
Gnomulus Thorell, 1890	2:2:2:2 2:2:3:3 (most)	Proximad	53
Caenoncopus Martens and Schwendinger, 1998	1:1:2:2 1:1:3:3 (most)	Proximad	3
Palaeoncopus Martens and Schwendinger, 1998	1:1:3:3	Distad	3
Biantoncopus Martens and Schwendinger, 1998	2:2:3:3	Distad	1
Martensiellus Schwendinger, 2006	1:1:2:2	Distad	1
gen. nov. sp. nov.	Unknown	Unknown	0

Table 2
List of species with MCZ accession numbers and GenBank® accession numbers for the eight markers employed in this study.

	MCZ Acc. #	18S rRNA	28S rRNA	H3	H4	U2	12S rRNA	16S rRNA	COI
Outgroups									
Peltonychia clavigera (Simon, 1879)	DNA101459	FJ796479		FJ796498	FJ475954	FJ475967			FJ796491
Equitius doriae Simon, 1880	DNA100607	U37003	EF108579	EF108595	FJ475955	FJ475968			EF108590
Cynortula granulata Roewer, 1912	DNA100332	FJ796480		FJ796499	FJ475956	FJ475969			FJ796492
Glysterus sp.	DNA101422	FJ796481		FJ796502	FJ475957	FJ475970	FJ475831	FJ796472	FJ796493
Dongmoa sp.	DNA101100	FJ796477	FJ796489	FJ796500	FJ475952	FJ475971		FJ796471	FJ475905
Ethobunus zalmoxiformis (Roewer, 1949)	DNA101424	FJ796478	FJ796490	FJ796501	FJ475953	FJ475972			FJ796494
Sandokanidae									
Biantoncopus sp.	DNA103288							FJ475867	FJ475920
Caenoncopus affinis Martens and Schwendinger, 1998	DNA102591	FJ475877	FJ475896	FJ475929	FJ475943	FJ475979		FJ475862	FJ475916
Caenoncopus cuspidatus Schwendinger, 1992	DNA102026	FJ475868	FJ475888	FJ475921	FJ475959		FJ475833	FJ475848	
Caenoncopus sp.	DNA102588	FJ475874	FJ475893	FJ475927	FJ475940		FJ475839	FJ475852	FJ475906
Caenoncopus sp.	DNA102590	FJ475876	FJ475895		FJ475942	FJ475978		FJ475861	FJ475915
Caenoncopus sp.	DNA102593	FJ475879	FJ475903	FJ475931	FJ475945	FJ475981	FJ475842	FJ475853	FJ475917
Caenoncopus sp.	DNA102595	FJ475882		FJ475934	FJ475948	FJ475982	FJ475845	FJ475865	FJ475918
gen. sp.	DNA102597	FJ475885	FJ475900	FJ475937	FJ475950			FJ475856	FJ475919
Gnomulus armillatus (Thorell,, 1891)	DNA102589	FJ475875	FJ475894	FJ475928	FJ475941	FJ475977	FJ475840	FJ475860	FJ475914
Gnomulus dalat (Schwendinger and Martens, 2006)	DNA101101	FJ796482	FJ796486	FJ796504				FJ796474	FJ796495
Gnomulus javanicus Schwendinger and Martens, 2002	DNA102030	FJ475872	FJ475891	FJ475925	FJ475963		FJ475837	FJ475851	
Gnomulus latoperculum Schwendinger and Martens, 2002	DNA102028	FJ475870	FJ475902	FJ475923	FJ475961	FJ475974	FJ475835	FJ475850	FJ475912
Gnomulus latoperculum Schwendinger and Martens, 2002	DNA102029	FJ475871	FJ475890	FJ475924	FJ475962	FJ475975	FJ475836	FJ475858	FJ475913
Gnomulus rostratus Thorell, 1890	DNA101102	FJ796483	FJ796487	FJ796503	FJ475965	FJ475987		FJ796473	
Gnomulus sp. (*armillatus* group)	DNA102587	FJ475873	FJ475892	FJ475926	FJ475939	FJ475976	FJ475838	FJ475859	
Gnomulus sp. (*rostratus* group)	DNA102592	FJ475878	FJ475904	FJ475930	FJ475944	FJ475980	FJ475841	FJ475863	
Martensiellus sp.	n/a	FJ796485						FJ796476	FJ796497
Palaeoncopus gunung Martens and Schwendinger, 1998	DNA102596-1	FJ475883	FJ475898	FJ475935	FJ475966	FJ475983	FJ475846	FJ475866	FJ475909
Palaeoncopus gunung Martens and Schwendinger, 1998	DNA102596-2	FJ475884	FJ475899	FJ475936	FJ475949	FJ475984		FJ475855	
Palaeoncopus sp.	DNA102027	FJ475869	FJ475889	FJ475922	FJ475960		FJ475834	FJ475849	FJ475911
Sandokan doriae (Thorell, 1876)	DNA102598	FJ475886	FJ475901	FJ475938	FJ475951	FJ475985	FJ475847	FJ475857	FJ475910
Sandokan malayanus (Schwendinger and Martens, 2004)	DNA100321	EF108575	EF108580	EF108596	FJ475958	FJ475973	FJ475832	EF108585	EF108591
Sandokan tiomanensis (Schwendinger and Martens, 2004)	DNA102594-1	FJ475880		FJ475932	FJ475946		FJ475843	FJ475854	FJ475907
Sandokan tiomanensis (Schwendinger and Martens, 2004)	DNA102594-2	FJ475881	FJ475897	FJ475933	FJ475947		FJ475844	FJ475864	FJ475908
Sandokan truncatus (Thorell, 1891)	DNA101099	FJ796484	FJ796488	FJ796505	FJ475964	FJ475986		FJ796475	FJ796496

anidae, the first such analysis conducted with molecular sequence data for a Laniatores family. We performed a phylogenetic analysis of sequence data derived from eight molecular loci. The study included representatives of the six described sandokanid genera, as well as a putative new genus from Thailand. From the molecular phylogenies obtained, we examined previous hypotheses of morphological character evolution and evaluated the molecular markers used in this study as predictors of laniatorid phylogeny.

2. Materials and methods

2.1. Species sampling

Specimens were collected by several individuals (mainly P.J. Schwendinger and A. Schultz over two collecting expeditions, 2003–2007) from leaf litter from sites throughout Southeast Asia, and by G.G. during a collecting trip to Sulawesi (ref. Appendix A). Twenty-five specimens (including fourteen described species) of Sandokanidae, comprising all described genera and one putative new genus, are represented in our analyses. Outgroup taxa consisted of a single exemplar from each of six laniatorid families, from specimens sequenced in our laboratory. The six outgroup taxa were selected to represent the breadth of laniatorid diversity (both superfamilies of "Insidiatores" and all superfamilies of Grassatores except Epedanoidea[2]). All specimens included in the study and their locality data are given in Appendix A.

[2] In general, laniatorid superfamily designations are tenuous and poorly understood. The superfamily nomenclature was loosely employed in this study to select outgroup taxa among major lineages of Laniatores.

2.2. Molecular methods

Molecular markers consisted of two nuclear ribosomal genes (complete 18S rRNA and a 2.2 kb fragment of 28S rRNA); two nuclear protein-encoding genes (histones H3 and H4); one nuclear non-coding gene (U2 snRNA); and three mitochondrial markers, two ribosomal (12S rRNA and 16S rRNA) and one protein-encoding (cytochrome *c* oxidase subunit I, henceforth COI). Many of these markers have proven informative in studies of arthropod phylogenies, including Opiliones and other arachnids (Colgan et al., 1998; Edgecombe et al., 2000; Giribet et al., 2001; Hormiga et al., 2003; Murienne et al., 2008; Prendini et al., 2003, 2005; Boyer et al., 2005, 2007b; Boyer and Giribet, 2007).

Total DNA was extracted from single legs of animals using Qiagen's DNEasy® Tissue Kit (Valenia, CA, USA) by incubating in lysis buffer overnight, as described by Boyer et al. (2005). Purified genomic DNA was used as a template for PCR amplification. The complete 18S rRNA (ca. 1.8 kb) was amplified in overlapping fragments, using primer pairs 1F–5R, 3F–18Sbi, and 18Sa2.0–9R (Giribet et al., 1996; Whiting et al., 1997). The fragment of 28S rRNA was amplified using the primer sets 28S D1F–28Srd4b, 28Sa–28Srd5b and 28Srd4.8a–28Srd7b1 (Park and Ó Foighil, 2000; Schwendinger and Giribet, 2005; Edgecombe and Giribet, 2006). Histone H3 was amplified using the primer pair H3aF–H3aR (Colgan et al., 1998). Histone H4 was amplified using the primer pair H4F2S–H4F2er (Pineau et al., 2005). U2 snRNA was amplified using the primer pair U2F–U2R (Colgan et al., 1998). 12S rRNA was amplified using the primer pair 12Sai–12Sbi (Kocher et al., 1989). 16S rRNA was amplified using the primer pair 16Sa–16Sb (Xiong and Kocher, 1991; Edgecombe et al., 2002). COI was amplified using the primer pair LCO1490–HCOoutout (Folmer et al.,

Table 3
List of primer sequences used for amplification and sequencing with original references of the primers sequences.

12S rRNA		
12Sai	5′-AAA CTA GGA TTA GAT ACC CTA TTA T-3′	Kocher et al. (1989)
12Sbi	5′-AAG AGC GAC GGG CGA TGT GT-3′	Kocher et al. (1989)
16S rRNA		
16Sa	5′-CGC CTG TTT ATC AAA AAC AT-3′	Xiong and Kocher (1991)
16Sb	5′-CTC CGG TTT GAA CTC AGA TCA-3′	Edgecombe et al. (2002)
18S rRNA		
1F	5′-TAC CTG GTT GAT CCT GCC AGT AG-3′	Giribet et al. (1996)
3F	5′-GTT CGA TTC CGG AGA GGG A-3′	Giribet et al. (1996)
5R	5′-CTT GGC AAA TGC TTT CGC-3′	Giribet et al. (1996)
9R	5′-GAT CCT TCC GCA GGT TCA CCT AC-3′	Giribet et al. (1996)
18Sa2.0	5′-ATG GTT GCA AAG CTG AAA C-3′	Whiting et al. (1997)
18Sbi	5′-GAG TCT CGT TCG TTA TCG GA-3′	Whiting et al. (1997)
28S rRNA		
28Sa	5′-GAC CCG TCT TGA AAC ACG GA-3′	Whiting et al. (1997)
28S D1F	5′-GGG ACT ACC CCC TGA ATT TAA GCAT-3′	Park and Ó Foighil (2000)
28Srd4b	5′-CCT TGG TCC GTG TTT CAA GAC-3′	Edgecombe and Giribet (2006)
28Srd5b	5′-CCA CAG CGC CAG TTC TGC TTA C-3′	Schwendinger and Giribet (2005)
28Srd4.8a	5′-ACC TAT TCT CAA ACT TTA AAT GG-3′	Schwendinger and Giribet (2005)
28Srd7b1	5′-GAC TTC CCT TAC CTA CAT-3′	Schwendinger and Giribet (2005)
COI		
LCO1490	5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′	Folmer et al. (1994)
HCOoutout	5′-GTA AAT ATA TGR TGD GCT C-3′	Prendini et al. (2005)
Histone H3		
H3aF	5′-ATG GCT CGT ACC AAG CAG ACV GC-3′	Colgan et al. (1998)
H3aR	5′-ATA TCC TTR GGC ATR ATR GTG AC-3′	Colgan et al. (1998)
Histone H4		
H4F2S	5′-TSC GIG AYA ACA TYC AGG GIA TCA C-3′	Pineau et al. (2005)
H4F2er	5′-CKY TTI AGI GCR TAI ACC ACR TCC AT-3′	Pineau et al. (2005)
U2 snRNA		
U2F	5′-TCT CGG CCT WWT GGC TAA-3′	Colgan et al. (1998)
U2R	5′-GMG GTA STG CAA TAC CGG-3′	Colgan et al. (1998)

1994; Prendini et al., 2005). Primer sequences are indicated in Table 3.

Polymerase chain reactions (PCR) (50 μL) consisted of 4 μL of template DNA, 1 μM of each primer, 200 μM of deoxynucleoside triphosphates (dNTPs; Invitrogen), 1X PCR buffer containing 1.5 mM $MgCl_2$ (Applied Biosystems, Branchburg, NJ, USA) and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems). The PCR reactions were carried out using a GeneAmp PCR System 9700 thermal cycler, and were comprised of an initial denaturation step (5 min at 95 °C), followed by 35 cycles including denaturation at 95 °C for 30 s, annealing at 49 °C for 30 s, and extension at 72 °C for 60 s, with a final extension step at 72 °C for 10 min.

Double-stranded PCR products were visualized by agarose gel electrophoresis (1% agarose) and purified using Qiagen QiaQuick spin columns. The purified PCR products were sequenced directly; each sequence reaction contained a total volume of 10 μL consisting of 3 μL of PCR product, 1 μM of one of the PCR primer pairs, 0.5 μL of ABI BigDye™ 55 sequencing buffer, and 1 μL of ABI BigDye™ Terminator v3.0 (Applied Biosystems). The sequence reactions, performed using the thermal cycler described above, involved an initial denaturation step for 3 min at 95 °C, and 25 cycles (95 °C for 10 s, 50 °C for 5 s, 60 °C for 4 min). The BigDye™-labeled PCR products were cleaned with AGTC® gel filtration cartridges or plates (Edge BioSystems, Galthesburg, MD, USA). The sequence reaction products were then analyzed using an ABI Prism 3730 genetic analyzer.

Chromatograms obtained from the automatic sequencer were read and sequences assembled using the sequence editing software Sequencher™ 4.7 (Gene Codes Corporation, Am Arbor, MI, USA). Sequence data were edited in MAC GDE 2.2 (Linton, 2005). All new sequences have been deposited in GenBank® under the Accession Nos. FJ475831–FJ475987 (see Table 2).

2.3. Phylogenetic analyses

Parsimony analysis was based on a direct optimization approach (Wheeler, 1996). DNA sequence data were analyzed in the computer package POY v. 4, build 2885 (Varón et al., 2008). Each gene was analyzed independently and in combination with all other molecular data. Tree searches were conducted by multiple cycles of (1) random addition sequences with subtree pruning and grafting (SPR), (2) tree bisection and reconnection (TBR), (3) ratcheting (Nixon, 1999), and (4) tree-fusing (Goloboff, 1999, 2002), on 24 CPUs of a cluster at Harvard University, Center for Genomic Research (portal.cgr.harvard.edu).

For parsimony, a parameter space of two variables (indel/transversion ratio and transversion/transition ratio) was explored, for a total of thirteen parameter sets analyzed per partition or combination of genes. We undertook a sensitivity analysis of the thirteen parameter sets (Wheeler, 1995), and used a modification of the incongruence length difference (ILD) as a criterion for selecting a favored parameter set. The sensitivity analysis included a parameter set designated 3221, which some propose represents a philosophical equivalent to unweighted parsimony (De Laet, 2005). Nodal support was estimated via jackknifing (1000 replicates) with a probability of deletion of e^{-1} (Farris et al., 1996; Farris, 1997). The data were analyzed for the optimal parameter set (411), in combination and for each independent partition.

Maximum likelihood (ML) analysis was performed using the GTR model of sequence evolution with corrections for a discrete gamma distribution and a proportion of invariant sites, (GTR + I + Γ) as selected in Modeltest v.3.7 (Posada, 2005; Posada and Crandall, 1998) under the Akaike information criterion (Posada and Buckley, 2004). As a starting point, we used the implied alignment from the optimal parameter set in the parsimony analysis (Wheeler, 2003; Giribet, 2005) for the combined data set and submitted the static alignment to RAxML v.7.0.0 (Stamatakis, 2006) on the CIPRES cluster, at the San Diego Supercomputation Center. Nodal support was estimated via bootstrapping (1000 replicates) (Felsenstein, 1985; Stamatakis et al., 2008).

Bayesian inference was performed using MrBAYES v.3.1 (Ronquist and Huelsenbeck, 2005) with the GTR + I + Γ model recommended by Modeltest under the Akaike information criterion, specified above. We used the default priors starting with random trees, and three heated and one cold Markov chains were run for 1,000,000 generations. After burn in samples were discarded, trees were combined in a single majority consensus topology, and the percentage of nodes were taken as posterior probabilities.

3. Results

Of the 13 parameter sets examined for the data, overall incongruence among partitions was minimized by parameter set 411 (indel:transversion = 4:1, transversion:transition = 1:1, ILD = 0.0341), the "optimal" parameter set for these data (Table 4). After repeated cycles of branch swapping, ratcheting and tree-fusing, the analyses found a single shortest tree of 5690 weighted steps, shown in Fig. 2. Sandokanidae was found monophyletic under all investigated parameter sets and received high nodal support (jackknife frequency 100%). The genera *Caenoncopus*, *Palaeoncopus* and *Sandokan* were also found monophyletic, receiving high nodal support frequencies (100% each) and proving stable under a variety of weighting schemes. The cladistic validity of *Gnomulus* is question-

Table 4
Tree lengths for different data partitions analyzed and congruence values (ILD) for the combined analysis of the eight molecular loci. Boldface marks the parameter set minimizing ILD value.

	18S rRNA	16S rRNA	12S rRNA	COI	H3	H4	U2	28S rRNA	MOL	ILD-MOL
111	183	698	467	1679	484	236	77	1031	5031	0.034983
121	252	1059	728	2446	681	333	104	1514	7393	0.037333
141	386	1750	1230	3907	1072	518	156	2454	11987	0.042880
181	642	3135	2230	6819	1848	886	260	4322	21162	0.048200
211	196	779	517	1709	484	236	77	1124	5303	0.034132
221	276	1208	818	2482	681	333	104	1692	7883	0.036661
241	427	2052	1400	3991	1072	518	156	2799	12952	0.041461
281	722	3740	2564	7001	1848	886	260	5009	23048	0.044169
411	**220**	**899**	**582**	**1720**	**484**	**236**	**77**	**1278**	**5690**	**0.034095**
421	317	1447	945	2500	681	333	104	1984	8638	0.037856
441	507	2528	3098	4024	1072	518	156	3378	14455	0.053707
481	882	4672	1663	7064	1848	886	260	6150	26066	0.101320
3221	380	1489	994	3399	968	472	154	2169	10379	0.034107

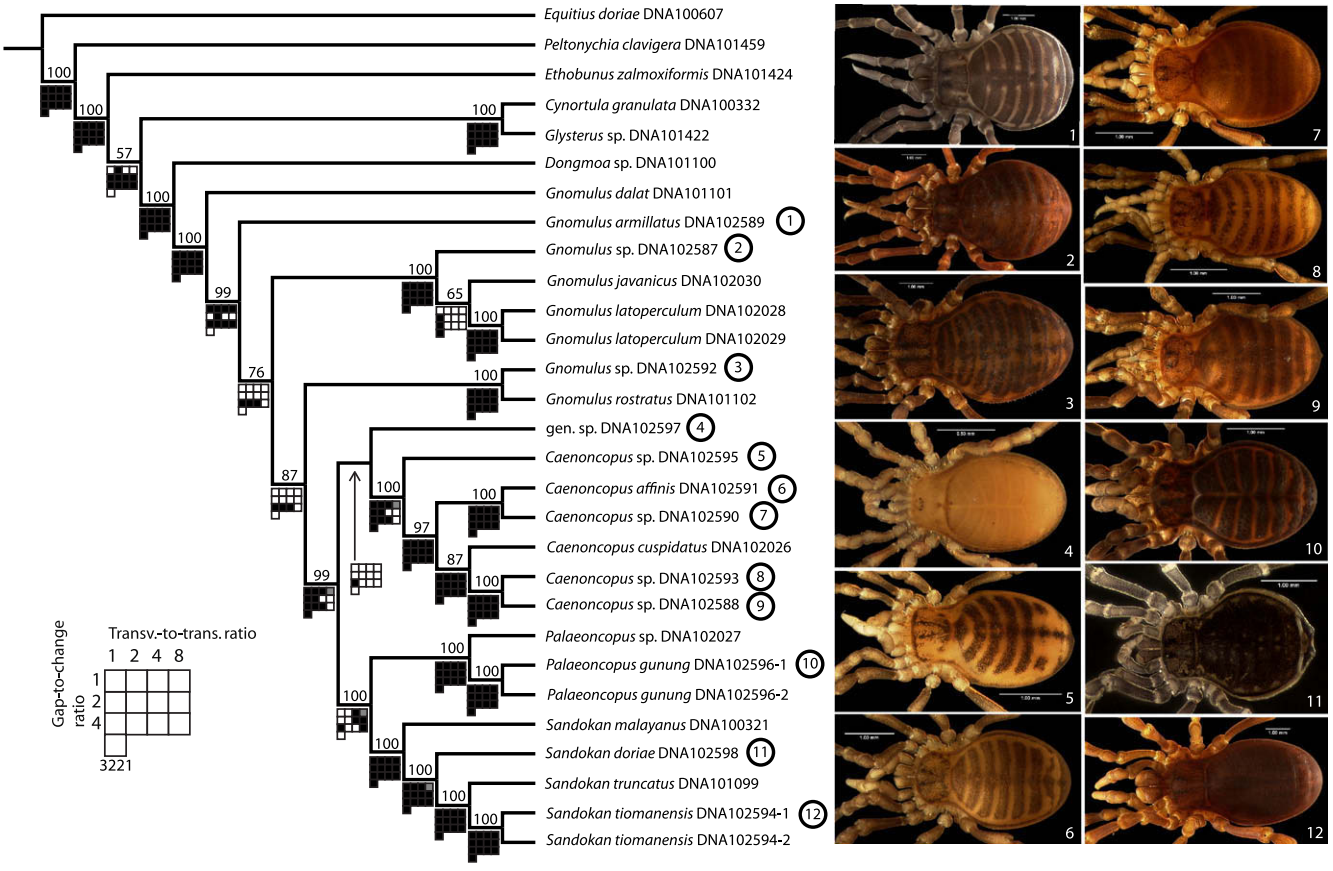

Fig. 2. Phylogenetic relationships of Sandokanidae with exclusion of *Biantoncopus* and *Martensiellus* (missing in over half of the data partitions), based on the single most parsimonious tree (parameter set 411, at 5690 weighted steps) of all data partitions. Numbers on branches indicate jackknife support values. Representative specimens are illustrated in dorsal view.

able, given that the combined analysis of all data partitions finds this genus paraphyletic, but particular subsets of the data (nuclear ribosomal genes, Fig. 3; non-coding genes, Fig. 4; nuclear genes, Fig. 5) consistently find it monophyletic and with high jackknife support values.

The monotypic genera *Biantoncopus* and *Martensiellus* were relatively poorly represented in the molecular sequence data partitions. Consequently, a separate parsimony analysis was conducted to determine their placement within Sandokanidae (Fig. 6). *Biantoncopus* was found sister to *Palaeoncopus*, and *Martensiellus* to the unidentified specimen (voucher specimen MCZ DNA102597).

The maximum likelihood analysis resulted in a tree topology with $\ln L = -30739.64$, and final parameters as follows: base frequencies $A = 0.247$, $C = 0.233$, $G = 0.269$, $T = 0.250$; $\alpha = 0.577$. The likelihood tree topology (Fig. 7) is most similar to the topology retrieved by parsimony analysis of non-coding data partitions (Fig. 4), with major differences being (1) the intrageneric relationships of *Gnomulus* and *Caenoncopus*, and (2) the relationships among outgroups. In contrast to parsimony analysis of all data partitions, ML analysis places the unidentified specimen ("gen. sp."), not *Gnomulus*, sister to the remaining Sandokanidae and retrieves monophyly of the large genus *Gnomulus*.

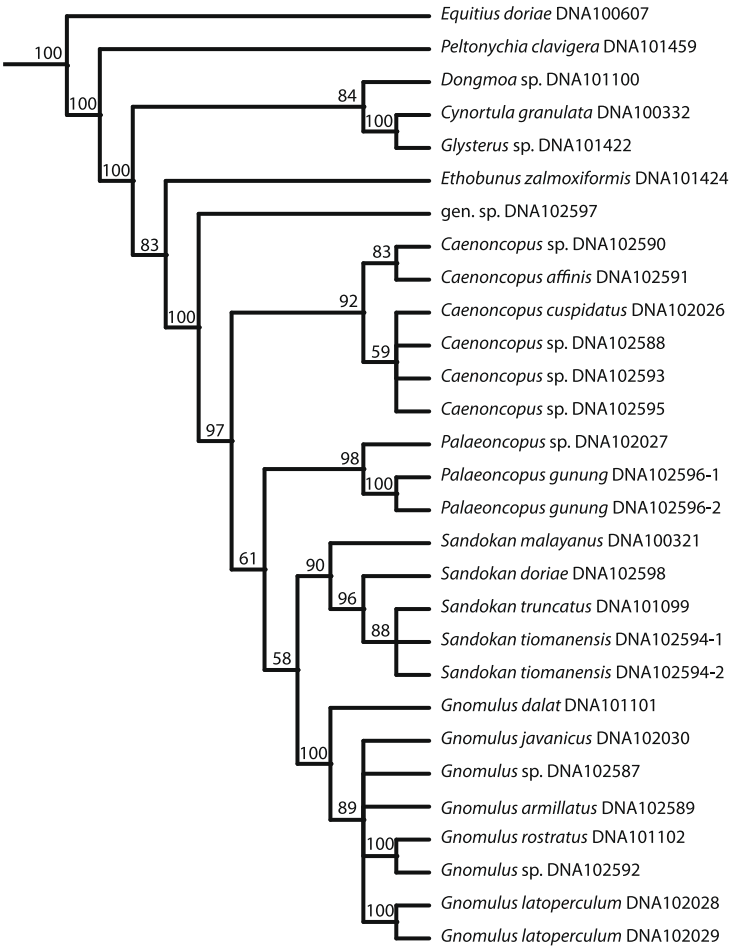

Fig. 3. Phylogenetic relationships of Sandokanidae (excluding *Biantoncopus* and *Martensiellus*) based on the strict consensus of 14 most parsimonious trees (parameter set 411, at 1510 weighted steps) of the nuclear ribosomal data partitions (18S rRNA + 28S rRNA). Numbers on branches indicate jackknife support values.

The runs of MrBAYES reached stationarity after ca. 240,000 generations; 250,000 generations were discarded as burn in. The Bayesian tree topology (Fig. 8) is nearly identical to the ML tree. Like the ML analysis, Bayesian inference places the unidentified specimen from Thailand sister to the remaining Sandokanidae, but differs in the intrageneric relationships of *Gnomulus*. In addition, Bayesian inference is unable to resolve the relationships among the derived sandokanid lineages, although these lineages are found monophyletic with high posterior probability. No relationship among the four sandokanid genera represented by multiple individuals is found to have significant posterior probability values.

4. Discussion

A series of recent revisions, facilitated by painstaking and extensive morphological studies, has shed much light on the diversity of Sandokanidae (Martens and Schwendinger, 1998; Schwendinger and Martens, 1999, 2002a, 2004, 2006; Schwendinger, 2006, 2007b). In addition to delimitation of generic boundaries, these studies also engendered explicit hypotheses of phylogeny and morphological character evolution (Martens and Schwendinger, 1998; Schwendinger and Martens, 2002b). The present study, with the inclusion of molecular sequence data, is principally aimed at testing the validity of the defined genera and investigating hypotheses of sandokanid evolution and biogeography stemming from morphological and molecular evidence.

4.1. Systematics of Sandokanidae

Our results indicate that Sandokanidae is a stable, strongly supported monophyletic group, an outcome that is in accordance with the distinct synapomorphies of this family, and furthermore has a similarly supported relationship to Podoctidae (Figs. 1 and 6). While the sampling of Laniatores is far from complete in this dataset, that the podoctid exemplar is consistently found sister to Sandokanidae is significant for two reasons. First, the sister group of Sandokanidae has historically been ambiguous (Schwendinger, 2007a) due to the highly autapomorphic nature of sandokanid morphology. Second, Podoctidae share much of their distribution with Sandokanidae, particularly in southeastern Asia, where podoctid diversity concentrates. Both families are restricted to the Old World (the "Cuban" genus *Ibantila* is introduced; Šilhavý, 1969; A. Pérez González, *personal communication*). The salient distinction is the broader range of Podoctidae, which are additionally found in Africa, the Mascarene Islands, the Seychelles, Australia, New Guinea, New Caledonia, and Vanuatu. The last of these comprises a group of volcanic islands between 3 and 15 Ma (Hall, 2002), and its inclusion in the range of Podoctidae is largely indicative of this taxon's dispersal ability, which may partially explain the comparatively greater range of podoctids.

Though the sister relationship of Podoctidae and Sandokanidae retrieved by our analyses accords with their overlapping distributions, more complete sampling of Podoctidae and other candidate taxa is necessary to establish definitively the sister group of San-

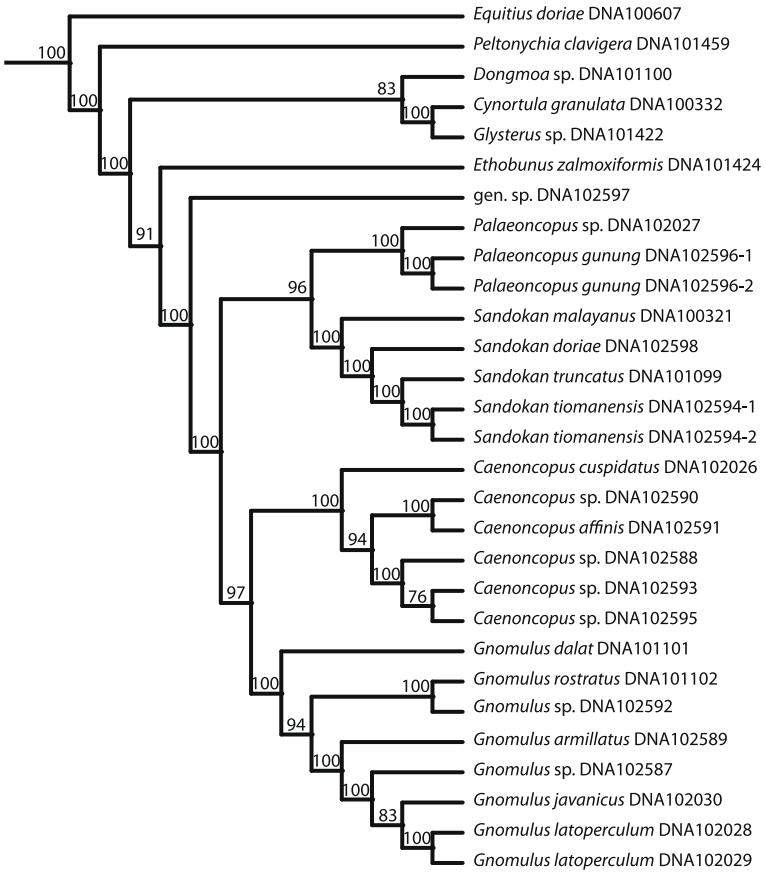

Fig. 4. Phylogenetic relationships of Sandokanidae (excluding *Biantoncopus* and *Martensiellus*) based on the single most parsimonious tree (parameter set 411, at 3121 weighted steps) of the non-protein-encoding data partitions (12S rRNA + 16S rRNA + 18S rRNA + 28S rRNA + U2 snRNA). Numbers on branches indicate jackknife support values.

dokanidae, given the large number of laniatorid families distributed in southeastern Asia (Epedanidae is conspicuously missing among the outgroup taxa). Results from a broader molecular phylogeny of Opiliones including most laniatorid families, but fewer markers, fail to corroborate the sister relationship of Podoctidae and Sandokanidae, although the two families together with Phalangodidae form a grade at the base of Grassatores (Giribet et al., submitted for publication).

Within Sandokanidae, three genera, *Sandokan* (formerly *Oncopus*), *Caenoncopus*, and *Palaeoncopus*, form well supported, stable groups, corroborating morphological studies and established synapomorphies. In a recent revision of *Sandokan*, Schwendinger and Martens (2004) proposed four species groups on the basis of genitalic morphology—the *feae*-group, the *truncatus*-group, the *doriae*-group, and the *hosei*-group—and postulated the following phylogeny: (*hosei*-group (*doriae*-group (*feae*-group + *truncatus*-group))). Our molecular sequence data suggest an alternative topology, with a sister relationship between the *doriae*-group (*S. doriae*) and the *truncatus*-group (*S. truncatus*, *S. tiomanensis*), and the *feae*-group (*S. malayanus*) sister to this clade. The *hosei*-group species were not represented in this study. The monophyly of *Sandokan* is hardly an unexpected result, given the unambiguous synapomorphies for the genus (reviewed in Schwendinger and Martens, 2004). In particular, the reduction of tarsomeres to a single article on all walking legs is unique to this genus among Laniatores.

Both *Caenoncopus* and *Palaeoncopus* are genera with little diversity (three described species each) endemic to Sumatra, and clearly distinguished from other Sandokanidae by aspects of male genitalic morphology. A number of undescribed *Caenoncopus* species are included in the analyses, but the resulting structure of the internal phylogeny does not support a local biogeographical signal; *Caenoncopus* from North Sumatra and West Sumatra cluster irrespective of collection localities, which is unusual among opilionid groups with limited dispersal ability (Boyer et al., 2007b; Sharma and Giribet, in press).

Gnomulus, the largest genus of Sandokanidae, dwarfs the other genera in diversity and geographical range. Apropos, it has received much attention in the form of taxonomic augmentations and revisions. Subsequent to the synonymy of *Pelitnus* with *Gnomulus* (Martens and Schwendinger, 1998), *Gnomulus* was iteratively subdivided into eleven species groups in conjunction with copious species descriptions (Schwendinger and Martens, 1999, 2002a, 2006). The present study poorly represents the diversity of *Gnomulus* species groups (four of the eleven), many of which are monotypic and comprise rare collections not available for molecular study. Parsimony analysis of combined data partitions suggests that *Gnomulus* is a paraphyletic grade that includes the remaining Sandokanidae (Figs. 2 and 6). However, reconstruction based on (1) nuclear ribosomal markers (18S rRNA + 28S rRNA), (2) the subset of markers that do not encode protein products (12S rRNA + 16S rRNA + 18S rRNA + 28S rRNA + U2 snRNA), or (3) nuclear markers (H3 + H4 + 18S rRNA + 28S rRNA + U2 snRNA) consistently yields a monophyletic, derived *Gnomulus* with high support (100% in all cases; Figs. 3–5, respectively).

Conflict between the coding gene COI (the only marker excluded in the above subsets) and the remaining partitions in this study results in artifactual paraphyly and placement of *Gnomulus*. Parsimony analysis of seven genes (all partitions excluding COI, fig-

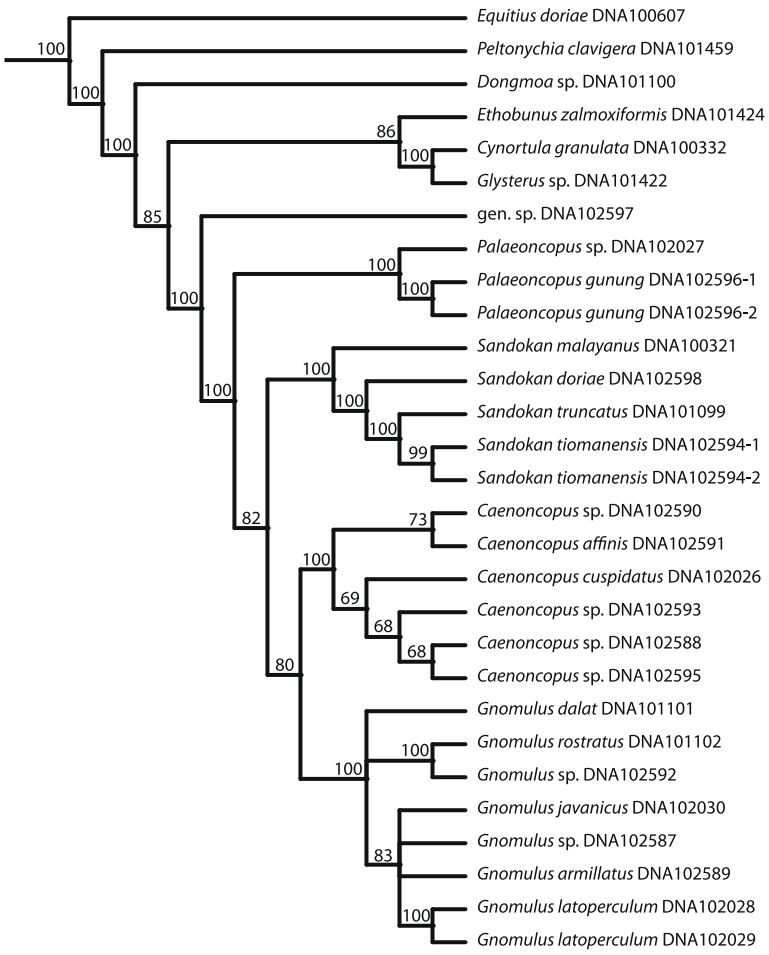

Fig. 5. Phylogenetic relationships of Sandokanidae (excluding *Biantoncopus* and *Martensiellus*) based on a strict consensus of the three most parsimonious trees (parameter set 411, at 2350 weighted steps) of the nuclear data partitions (histone H3 + histone H4 + 18S rRNA + 28S rRNA + U2 snRNA). Numbers on branches indicate jackknife support values.

ure not shown) yields a topology nearly identical to that retrieved by nuclear markers (Fig. 5). Missing data for *Gnomulus* in the COI data set (ref. Table 2), or a rooting problem caused by the distance to its nearest outgroup (e.g., Wheeler, 1990; Giribet et al., 2005), may also contribute to the retrieved paraphyly. In contrast, maximum likelihood and Bayesian analyses based on all data partitions consistently yield a monophyletic *Gnomulus* (Figs. 7 and 8), as a derived group in the case of the ML analysis. A conclusive analysis of this group's validity, as well as determination of relationships among the eleven phyletic lineages designated within the genus (Schwendinger and Martens, 2002a, 2006), requires a broader sampling of constituent species with reevaluation of molecular markers suitable for this purpose.

Conflict among protein-encoding and non-coding genes is not infrequent, as shown recently in a densely sampled phylogeny of centipedes including both sets of markers (Giribet and Edgecombe, 2006). Conflict among families of protein-encoding genes has also been documented in recent phylogenomic and genome-wide analyses (e.g., Rokas et al., 2003). Whether the conflicting markers are still useful for reconstructing phylogeny is still debatable, but it seems that hidden support may be an important phenomenon (Gatesy and Baker, 2005; Rokas et al., 2003).

4.2. Intergeneric relationships

Sandokan was proposed to hold an advanced position in sandokanid phylogeny, either as the sister group to *Gnomulus* or *Caenoncopus* (Schwendinger and Martens, 2004). Specifically, sexual dimorphism in the carapace and chelicera, and aspects of genitalic morphology were proposed to unite *Sandokan* and *Gnomulus* (Schwendinger and Martens, 2004). Our results for the parsimony direct optimization analysis of all markers corroborate the derived position of *Sandokan* in the phylogeny, but its sister group was found to be *Palaeoncopus*. The result is unexpected from a morphological perspective because *Palaeoncopus* is distinguished by (and named for) its "primitive" genitalic morphology (Martens and Schwendinger, 1998); in a study of genitalic evolution in Sandokanidae, Schwendinger and Martens (2002b) explicitly hypothesized that *Gnomulus*, *Caenoncopus*, and *Sandokan* represented derived states of both genitalic characters and tarsomere count, *Biantoncopus* the intermediate states, and *Palaeoncopus* the ancestral states (Fig. 9a). The results from parsimony analysis of all data partitions suggest a near-inversion of the Schwendinger and Martens (2002b) reconstruction, with the derived *Sandokan* and *Palaeoncopus* clustering with *Caenoncopus* and a putative new genus, and *Gnomulus* representing the lineage(s) sister to the remaining Sandokanidae. By contrast, ML analysis supports most elements of the Schwendinger and Martens reconstruction, namely, the derived position and monophyly of *Gnomulus* (Fig. 7).

The relationships of these genera are somewhat subject to change when altering parameter sets. The ML and Bayesian tree topologies in particular show no strongly supported relationships among the described genera, barring the (*Sandokan* + *Palaeoncopus*) clade with moderate (86% bootstrap frequency) support in the ML

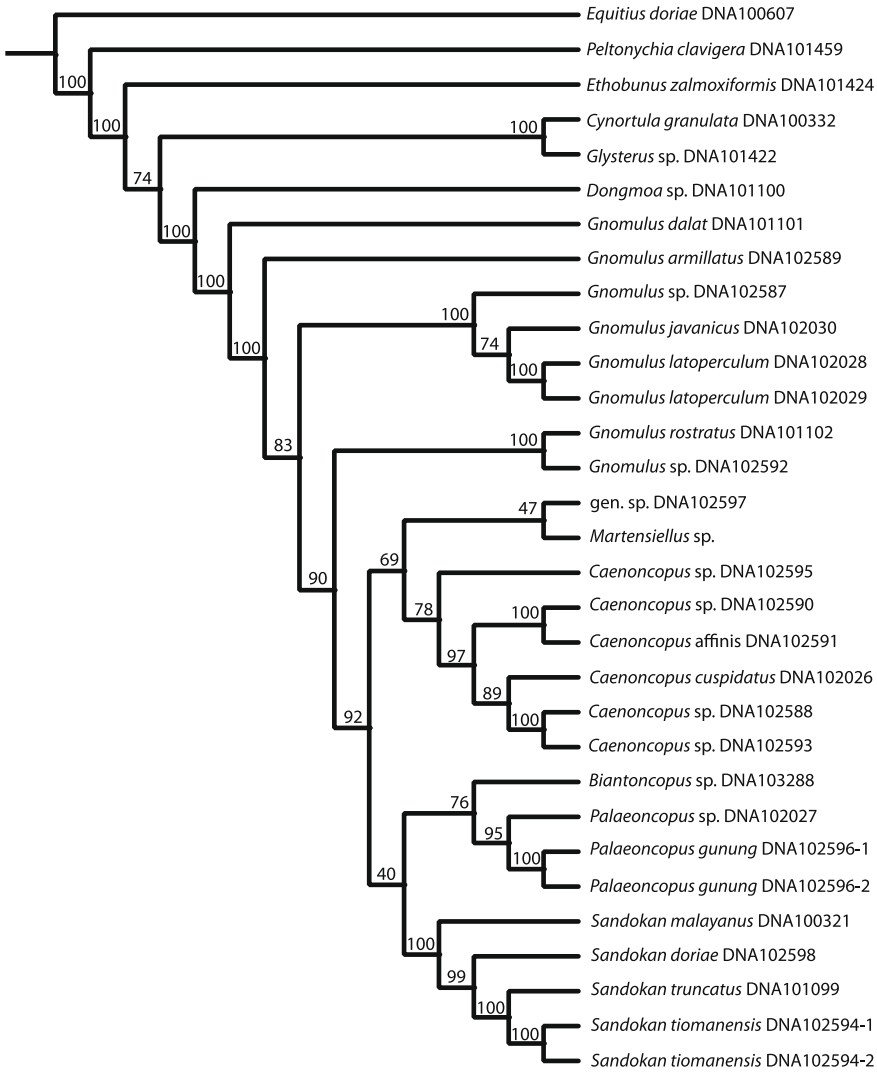

Fig. 6. Phylogenetic relationships of Sandokanidae based on the single most parsimonious tree (parameter set 411, 5929 weighted steps) of all data partitions and including all taxa. Numbers on branches indicate jackknife support values.

analysis (Figs. 7 and 8). Consequently, we examined the behavior of parsimony tree topology under the seven parameter sets that discorded with the resolution of a derived (*Sandokan* + *Palaeoncopus*) clade. Two of these parameter sets (211 and 3221) favor an apical genus-level topology identical to that retrieved by the optimal parameter set (*Caenoncopus* (*Sandokan* + *Palaeoncopus*)), differing only in the placement of the unidentified genus, ("gen. sp."). Another two sets (421 and 441) favor the topology (*Palaeoncopus* (*Caenoncopus* (gen. sp. + *Sandokan*))). The remaining three (111, 121, and 221) favor the topology (*Sandokan* (*Palaeoncopus* + *Caenoncopus*)), with some variation in the placement of the new genus. Under all parameter sets, *Gnomulus*, not *Palaeoncopus*, is placed sister to the rest of Sandokanidae. In addition, the same three partitions that consistently yield a monophyletic, derived *Gnomulus* (nuclear ribosomal genes, non-coding genes, and nuclear genes; Figs. 3–5), as well as maximum likelihood and Bayesian analyses (Figs. 7 and 8), also find the unidentified genus to be sister to the remaining sandokanids. Contrary to previous reconstructions of sandokanid evolution, therefore, *Palaeoncopus* represents a derived lineage of Sandokanidae.

The two monotypic genera *Biantoncopus* and *Martensiellus* were represented in this study by comparatively little molecular sequence data and their placement is therefore far from definitive. However, it was previously observed from genitalic morphology that *Biantoncopus* resembled *Palaeoncopus* (Martens and Schwendinger, 1998), and from somatic morphology that *Martensiellus* was likely related to *Caenoncopus* and *Palaeoncopus* (Schwendinger, 2006). These observations accord with the placement of *Biantoncopus* sister to *Caenoncopus*, and *Martensiellus* clustering with *Caenoncopus* (in addition to the unidentified specimen), after extensive parsimony searches, albeit with limited support (Fig. 6). Addition of molecular sequence data would do much to clarify the relationships of these two genera.

The unidentified sandokanid ("gen. sp.") proves problematic in its placement, a curious outcome given its representation in nearly all of the molecular data partitions (six of eight genes). Morphologically distinct from other genera (P.J. Schwendinger, *personal communication*), this taxon's ambiguous placement as sister to *Martensiellus* and/or *Caenoncopus* in the parsimony direct optimization analyses receives little support and is unstable (Figs. 2 and 6). Various parameter sets alternatively place it sister to *Palaeoncopus* (111, 121, 211, 3221), *Sandokan* (421, 441), or some combination of all three. The specimen therefore appears to represent a new genus of uncertain affinity, but it consistently clusters with the clade containing *Caenoncopus*, *Sandokan*, and *Palaeoncopus* in parsimony analyses. Alternatively, the specimen may represent

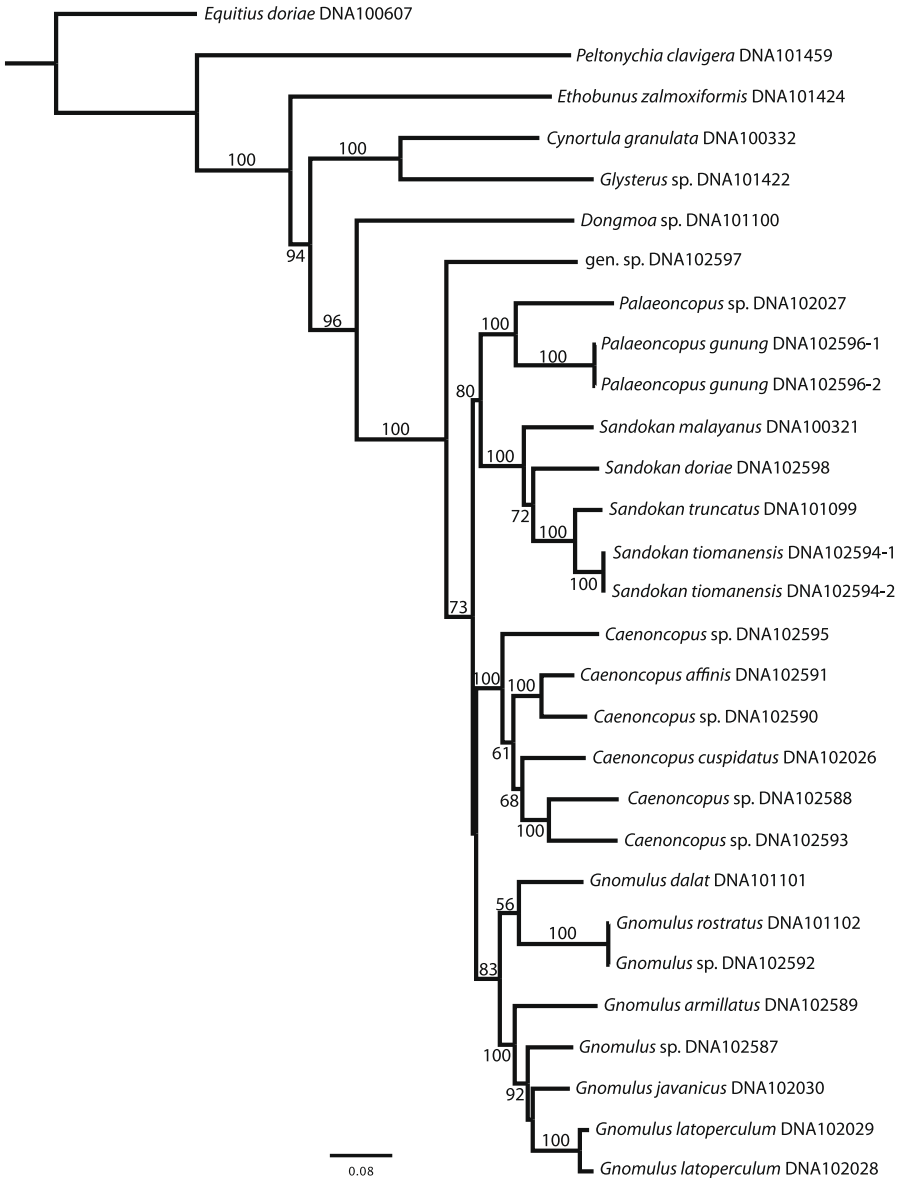

Fig. 7. Phylogenetic relationships of Sandokanidae inferred from maximum likelihood analysis of all molecular data ($\ln L = -30739.64$). Numbers on branches indicate bootstrap support values.

the lineage sister to the remaining Sandokanidae, as inferred by the probabilistic methods, as well as parsimony analyses of particular partitions (discussed above). If so, ongoing morphological study of this new genus may heavily influence understanding of sandokanid evolution.

4.3. Morphological evolution

Previously hypothesized relationships between sandokanid genera and the accompanying reconstruction of ancestral states have been principally guided by genitalic morphology, although the number of tarsal articles was also given significance (Schwendinger and Martens, 2002b). In our parsimony analysis, the placement of *Gnomulus* as sister to the derived Sandokanidae, as well as the clustering of *Palaeoncopus* with *Sandokan* at the apex of the phylogeny, suggests that the interpretation of these character states should be inverted, i.e., the plesiomorphic states are the ones previously considered derived. However, the reconstruction of sandokanid evolution by Schwendinger and Martens (2002b) is supported to varying extent by parsimony analyses of particular data partitions, and by ML and Bayesian analyses (discussed above). The phylogeny based on all nuclear genes, in particular, is in full accordance with the reconstruction by Schwendinger and Martens (2002b), barring the placement of *Biantoncopus*, which is almost unrepresented in the nuclear partition (ref. Table 2).

We mapped tarsomeric and genitalic characters of extant lineages onto schematized phylogenies obtained from parsimony (Fig. 9b) and ML (Fig. 9c) analyses; character evolution hypothesized by Schwendinger and Martens (2002b) is shown for comparison (Fig. 9a). Reconstruction based on the parsimony analysis shows a trend of decreasing tarsal articles, particularly in the first and second walking legs. Male genitalic morphology appears to have undergone multiple secondary reversals. The proximad-directed glans appears to be a plesiomorphic condition that may have been secondarily lost in *Martensiellus* and/or the new genus, and in the (*Palaeoncopus* + *Biantoncopus*) lineage. This character, as well as other genitalic characters (ref. Schwendinger and Martens, 2002b) that are highly variable within *Gnomulus* and among

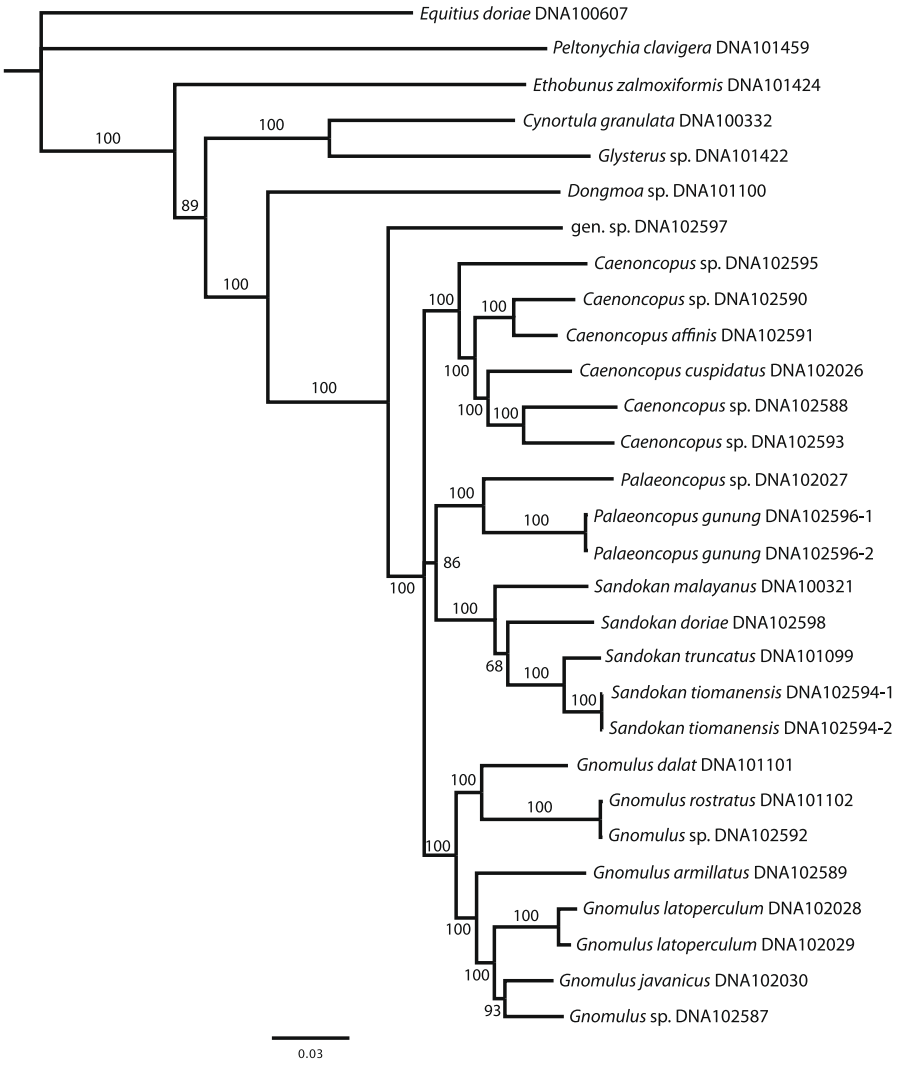

Fig. 8. Phylogenetic relationships of Sandokanidae inferred from Bayesian analysis of all molecular data. Numbers on branches indicate posterior probabilities.

Sandokanidae, recapitulates the usefulness of genitalic morphology for defining genera and determining their relationships.

Tarsal formula, traditionally used extensively and often erroneously to delimit laniatorid genera (e.g., Roewer, 1923), demonstrates an interesting evolutionary history in Sandokanidae as inferred by parsimony. Contrary to previous hypotheses (Schwendinger and Martens, 2002b), the plesiomorphic state for tarsal formula is 2:2:2–3:2–3, with a trend toward decreasing tarsalia among derived lineages (Fig. 9b). Additional tarsomeres of the first and second walking legs may have been reacquired in *Biantoncopus*, but the uncertain placement of this genus in this study renders this observation speculative. Martens and Schwendinger (1998) expressed uncertainty over the affinities of *Biantoncopus*, which resembles *Gnomulus* in somatic morphology, but also *Palaeoncopus* in genitalic morphology. Further investigation and future placement of *Biantoncopus* sister to *Gnomulus* would obviate the hypothesis of secondary gains in tarsomere number.

Reconstruction based on the ML phylogeny (Fig. 8c) corroborates at least one secondary reversal of glans direction in the *Palaeoncopus* lineage. Contrary to the reconstruction based on parsimony analysis, the topology supported by ML suggests that the plesiomorphic state for tarsal formula may be 1:1:3:3, with opposing trends toward increasing tarsalia on the first two pairs of walking legs in the *Gnomulus* lineage, and decreasing tarsalia on the second two pairs of walking legs in *Sandokan*. Owing to (1) the exclusion of *Biantoncopus* and *Martensiellus*, and (2) the lack of morphological studies of the new genus, it is presently unfeasible to reconstruct further genitalic or tarsomeric character state changes in the basal lineages of the ML tree.

4.4. Molecular markers and laniatorid phylogenies

A number of studies has validated the effectiveness of five molecular markers (the "workhorses") commonly utilized in arthropod systematics: cytochrome *c* oxidase subunit I (COI), 16S rRNA, 18S rRNA, 28S rRNA, and histone H3 (e.g., Balke et al., 2007; Boyer et al., 2005, 2007b; Giribet et al., 1999, 2002; Hormiga et al., 2003; Murienne et al., 2008; Prendini et al., 2003, 2005). However, recent progress in opilionid phylogeny has revealed shortcomings in the use of these markers for problematic taxa (Boyer and Giribet, 2007; Boyer et al., 2007a, 2007b), and especially for the family-level resolution of Laniatores (Giribet et al., submitted for publication). In this study, we utilized an additional three markers (U2 snRNA, 12S rRNA, and histone H4) to elucidate the relationships of a laniatorid family, and evaluated their performance by comparing the "principal" phylogeny (obtained from parsimony analysis of all eight markers and excluding taxa represented in less than half the data partitions; Fig. 2) with a phylogeny

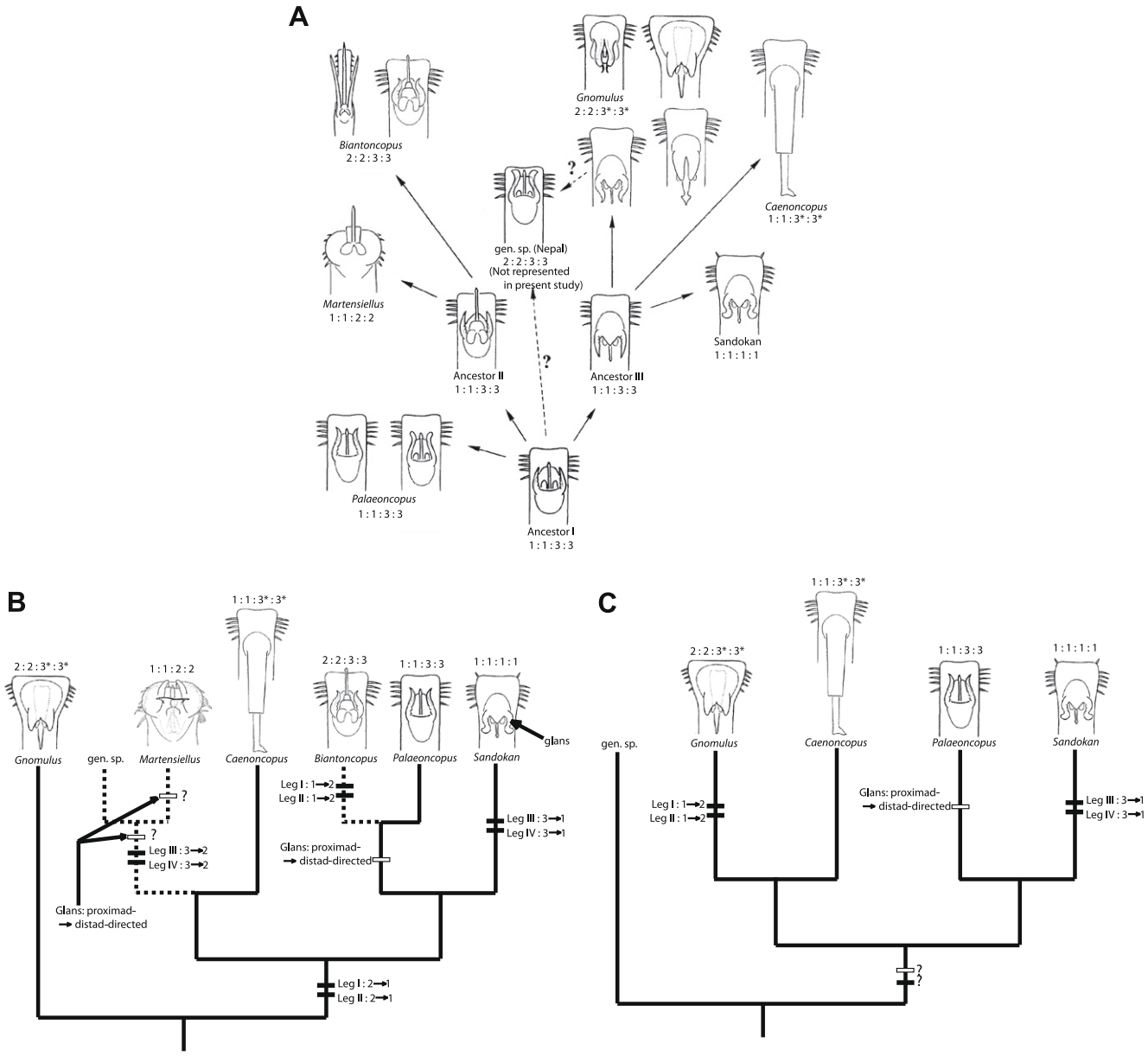

Fig. 9. (A) Intergeneric relationships of Sandokanidae postulated by Schwendinger and Martens (2002b) on the basis of genitalic and tarsomeric morphology. (B) Cladogram of hypothetical evolution of significant morphological characters in Sandokanidae, superimposed upon a schematized molecular phylogeny based on parsimony analysis of all data partitions. (C) Cladogram of hypothetical evolution of significant morphological characters in Sandokanidae, superimposed upon a schematized molecular phylogeny based on ML analyses of all data partitions. The ratios above the terminals are tarsal formulas, defined as the number of tarsal articles on the walking legs of adults, from leg I to leg IV; asterisks indicate variability in tarsalia number (2–3 tarsalia). Dashed lines indicate uncertain placement of genera. Filled rectangles denote genitalic character state changes. Open rectangles denote tarsomeric character state changes. Illustrations reprinted from Schwendinger and Martens (2002b) with the permission of the lead author and The Journal of Arachnology.

comprising identical terminals and optimality criterion, but estimated using the five commonly utilized markers. Two of these three markers (U2 snRNA and 12S rRNA) have been utilized for numerous arthropod groups, but not for internal Opiliones phylogenies (e.g., Colgan et al., 1998; Giribet et al., 2001, 2005; Prendini et al., 2003, 2005).

The phylogeny based on parsimony analysis of five markers (Fig. 10) shares salient characteristics with the eight-marker phylogeny; relationships among the outgroup taxa are unchanged, and those sandokanid genera found monophyletic in the eight-marker phylogeny are also found monophyletic with five data partitions. The critical differences include the relationships among the derived genera; the five-marker analysis places the new genus sister to *Palaeoncopus*, with this clade in turn sister to *Caenoncopus*. *Gnomulus* remains a paraphyletic grade, but with more lineages clustering together.

These differences in the two phylogenies in conjunction with the support measures suggest that the scale of resolution of the added markers (H4, U2, 12S) affects the relationships among and within genera, a desirable result, given that parts of other opilionid data sets require improvement at this scale (Boyer and Giribet, 2007; Clouse and Giribet, 2007). The support levels and stability of most sandokanid genera and species encourage continued use of the eight markers used in this study. However, the added markers have shortcomings, typified by the placement of the new genus in the parsimony analyses (Figs. 2 and 10). Similarly, the "paraphyly" of *Gnomulus*, likely an artifact of conflicting data partitions, similarly highlights putative character incongruence contributed

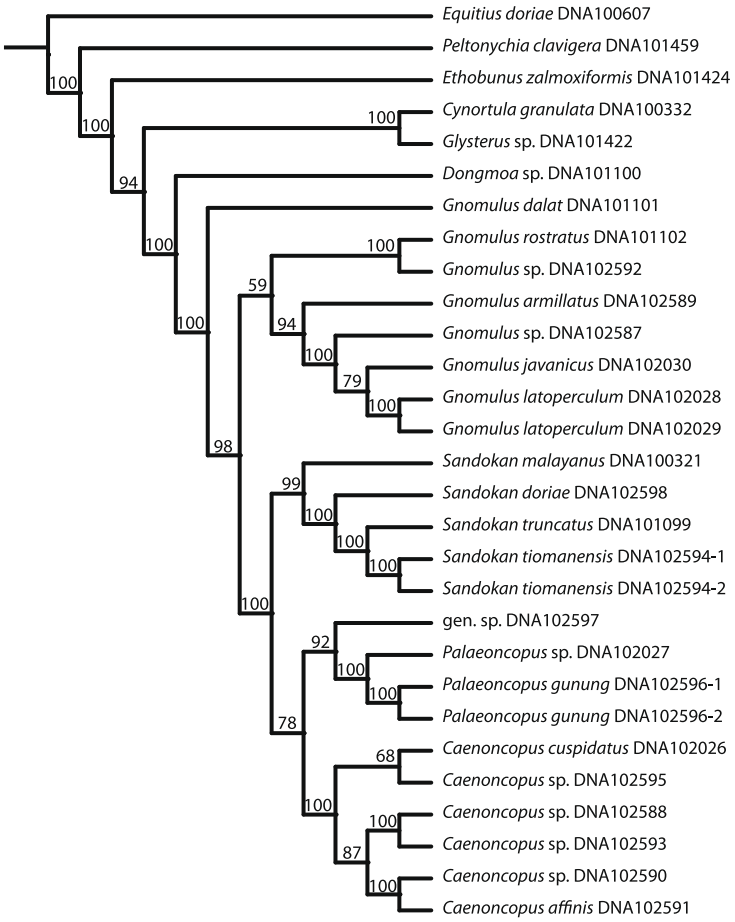

Fig. 10. Phylogenetic relationships of Sandokanidae (excluding *Biantoncopus* and *Martensiellus*) based on the single most parsimonious tree (parameter set 411, at 4737 weighted steps) of the five data partitions commonly utilized in arthropod systematics (16S rRNA + 18S rRNA + 28S rRNA + COI + H3). Numbers on branches indicate jackknife support values.

by the added markers that proves problematic for parsimony. Moreover, these markers appear to have little effect on the resolution of interfamilial relationships, a problem that plagues Laniatores phylogeny (Giribet et al., submitted for publication). Further improvement of laniatorid phylogenetic estimation necessitates continued investigation of molecular markers effective at a scale of resolution spanning interfamilial and intergeneric levels. Conserved loci, particularly nuclear coding genes, may prove more informative at this scale in future studies of Laniatores.

4.5. Biogeography of Sandokanidae

The distribution of Sandokanidae appears to be governed principally by limitations of this group's dispersal ability. Four other laniatorid families—Assamiidae, Epedanidae, Podoctidae, and Zalmoxidae—are distributed throughout Sundaland, but all of these have greater range than Sandokanidae, and frequently demonstrate clear dispersal events (Giribet and Kury, 2007). The restriction of Sandokanidae to Sundaland and the Philippines is suggestive of diversification in accordance with breakup of Sundaland's components.

Biogeographical hypotheses supported by results of phylogenetic analyses were visualized as area cladograms (Fig. 11) to elucidate the diversification of Sandokanidae. The area cladogram derived from parsimony analysis, which places the "paraphyletic" *Gnomulus* sister to the remaining Sandokanidae, suggests a southward pattern of cladogenesis, with ancestral lineages represented in the Thai-Malay Peninsula, and derived lineages occupying (and often restricted to) the constituent islands of the Archipelago. Sumatra includes at least two independent, endemic lineages, *Caenoncopus* and *Palaeoncopus*. Borneo is inhabited by *Sandokan* (which also inhabits the southern Malay Peninsula), and the endemic genus *Martensiellus*, but uncertainty in the placement of *Martensiellus* obscures interpretation of Borneo's role in sandokanid diversification.

In spite of an alternative topology of intergeneric relationships, ML and Bayesian analyses corroborate the southward pattern of cladogenesis, as the lineage sister to the remaining sandokanids (in this case, the new genus) always occupies the Thai-Malay Peninsula. The Malay Archipelago is occupied by derived lineages, excepting *Gnomulus*, which ranges from the mainland through the archipelago. In these reconstructions, *Gnomulus* appears to exhibit a microcosm of the southward expansion characteristic of the entire family; the lineage sister to the "derived" *Gnomulus* distributed in the Archipelago also occupies the Thai-Malay Peninsula (specifically, Vietnam and Thailand).

Analysis of sandokanid biogeography on the basis of our data is unfortunately limited by incomplete geographical sampling. Specifically, the broad range of *Gnomulus* in continental southeast Asia is poorly represented, as are the Philippine Islands, which are inhabited by at least two sandokanid genera (*Gnomulus* and *Biantoncopus*; Martens and Schwendinger, 1998). The biogeography of Sandokanidae also continues to be informed by ongoing collecting efforts and identifications of areas previously unknown to be occupied by particular genera (Schwendinger, 2007b; e.g., unidentified specimen in the present study).

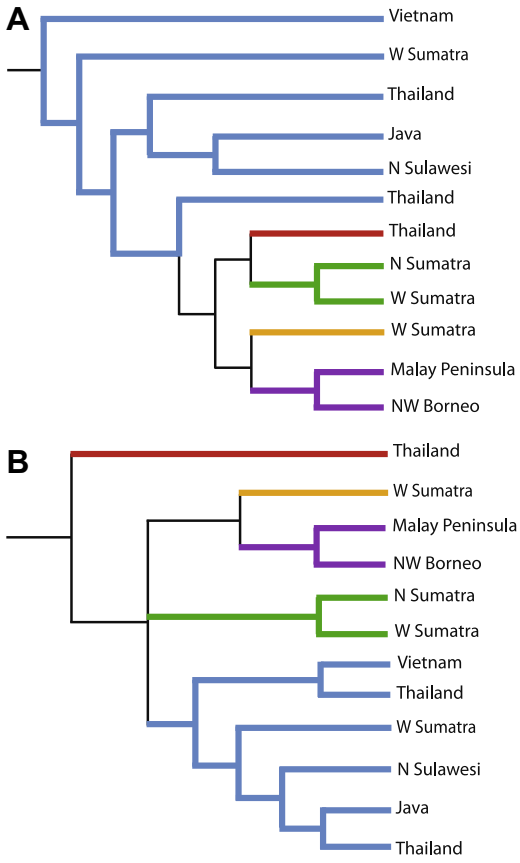

Fig. 11. (A) Area cladogram summarizing distributions of Sandokanidae (excluding *Biantoncopus* and *Martensiellus*) based on parsimony analysis of all data partitions. (B) Area cladogram summarizing distributions of Sandokanidae (excluding *Biantoncopus* and *Martensiellus*) based on parsimony analysis of nuclear data partitions (histone H3 + histone H4 + 18S rRNA + 28S rRNA + U2 snRNA). Colors correspond to lineages as follows: blue = *Gnomulus*, red = gen. nov. sp. nov., green = *Caenoncopus*, yellow = *Palaeoncopus*, purple = *Sandokan*.

Sandokanid distribution in the Philippine Islands is particularly informative about possible dynamics of diversification in this group. Sandokanidae have been reported from the islands Luzon, Mindanao and Palawan (various *Gnomulus* species), as well as Leyte (*Gnomulus* and *Biantoncopus*). This component of the distribution is curious insofar as only Palawan has a continental origin, whereas the other Philippine Islands are volcanic and of oceanic origin (Hall, 2002). The colonization of multiple volcanic islands in the Philippines suggests that some sandokanid lineages are capable of short-range, transoceanic dispersal, which may have influenced diversification in concert with continental fragmentation, particularly in the case of *Biantoncopus*, the monotypic genus endemic to Leyte.

The relevance of opilionid systematics to studies of historical biogeography is underscored by the similarities between Sandokanidae and the cyphophthalmid family Stylocellidae, which share a nearly identical distribution. Cyphophthalmi are demonstrably poor dispersers that have diversified in accordance with vicariant events (Boyer et al., 2007b), but Stylocellidae are unusual because they are found in western New Guinea, a distribution that almost certainly involved multiple transoceanic dispersal events and conflicts with "Lydekker's Line," the faunal break between the Molluccas and New Guinea that defines the eastern limit of poor dispersers from Eurasia (Lydekker, 1915; Smith, 1943; Mayr, 1953; George, 1981; Clouse and Giribet, 2007). Moreover, in spite of documented dispersal ability among the New Guinean exemplars, Stylocellidae of the Philippines are restricted to Palawan, in contrast to Sandokanidae. Nevertheless, biogeographical analyses of Stylocellidae in multiple datasets support a southward pattern of cladogenesis similar to Sandokanidae, with ancestral lineages occupying the Thai-Malay Peninsula (Boyer et al., 2007b; Clouse and Giribet, 2007). The correlation between the ranges of constituent genera (or species groups) of the two families is particularly striking. However, to our knowledge, no sandokanid has been reported from New Guinea.

The limited dispersal ability and ancient diversifications of these families, in conjunction with ongoing phylogenetic efforts, suggest that Opiliones may prove excellent candidates for studies of cladistic biogeography in biodiversity hotspots. The biogeography of Sundaland, in particular, may be further illuminated by investigation of other laniatorid families with varying dispersal abilities distributed throughout Sundaland and Wallacea, such as Podoctidae, Assamiidae, and Zalmoxidae.

Acknowledgments

We are wholly indebted to Peter Schwendinger, who collected, identified, and provided most of the material for study, and whose dedication to opilionid systematics has inspired much of our efforts. This work could have not been possible without his generosity and unconditional support. Ron Clouse and Cahyo Rahmadi provided support and good company during a collecting trip to Sulawesi, which was generously funded by a Putnam Expedition Grant from the Museum of Comparative Zoology, Harvard University. Support for sequencing came from the FAS Center for Genomic Research. Mike Ethier and the Life Sciences IT group kindly assisted with cluster operations. Discussions with Ron Clouse developed some of the ideas presented in this article. Nestor Ardila assisted with RAxML and other phylogenetic software. Associate Editor Stefan Richter and two anonymous reviewers made comments that helped to improve an earlier version of this manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2009.03.013.

References

Balke, M., Pons, J., Ribera, I., Sagata, K., Vogler, A.P., 2007. Infrequent and unidirectional colonization of hyperdiverse *Papuadytes* diving beetles in New Caledonia and New Guinea. Mol. Phylogenet. Evol. 42, 505–516.

Boyer, S.L., Karaman, I., Giribet, G., 2005. The genus *Cyphophthalmus* (Arachnida, Opiliones, Cyphophthalmi) in Europe: a phylogenetic approach to Balkan Peninsula biogeography. Mol. Phylogenet. Evol. 36, 554–567.

Boyer, S.L., Baker, J.M., Giribet, G., 2007a. Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread "mite harvestman" defies DNA taxonomy. Mol. Ecol. 16, 4999–5016.

Boyer, S.L., Clouse, R., Benavides, L., Sharma, P., Schwendinger, P., Karunarathna, I., Giribet, G., 2007b. Biogeography of the world: a case study of globally distributed arachnids. J. Biogeogr. 34, 2070–2085.

Boyer, S.L., Giribet, G., 2007. A new model Gondwanan taxon: systematics and biogeography of the harvestman family Pettalidae (Arachnida, Opiliones, Cyphophthalmi), with a taxonomic revision of genera from Australia and New Zealand. Cladistics 23, 337–361.

Clouse, R.M., Giribet, G., 2007. Across Lydekker's line—first report of mite harvestmen (Opiliones: Cyphophthalmi: Stylocellidae) from New Guinea. Invertebr. Syst. 21, 207–227.

Colgan, D.J., McLauchlan, A., Wislon, G.D.F., Livingston, S., Macaranas, J., Edgecombe, G.D., Cassis, G., Gray, M.R., 1998. Molecular phylogenetics of the Arthropoda: relationships based on histone H3 and U2 snRNA DNA sequences. Aust. J. Zool. 46, 419–437.

Curtis, D.J., Machado, G., 2007. Ecology. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, MA, pp. 280–285.

De Laet, J.E., 2005. Parsimony and the problem of inapplicables in sequence data. In: Albert, V.A. (Ed.), Parsimony, Phylogeny, and Genomics. Oxford University Press, Oxford, pp. 81–116.

Edgecombe, G.D., Giribet, G., Wheeler, W.C., 2002. Phylogeny of Henicopidae (Chilopoda: Lithobiomorpha): a combined analysis of morphology and five molecular loci. Syst. Entomol. 27, 31–64.

Edgecombe, G.D., Giribet, G., 2006. A century later—a total evidence re-evaluation of scutigeromorph centipedes (Myriapoda: Chilopoda). Invertebr. Syst. 20, 503–525.
Edgecombe, G.D., Wilson, G.D.F., Colgan, D.J., Gray, M.R., Cassis, G., 2000. Arthropod cladistics: combined analysis of histone H3 and U2 snRNA sequences and morphology. Cladistics 16, 155–203.
Farris, J.S., Albert, V.A., Källersjö, M., Lipscomb, D., Kluge, A.G., 1996. Parsimony jackknifing outperforms neighbor-joining. Cladistics 12, 99–124.
Farris, J.S., 1997. The future of phylogeny reconstruction. Zool. Scr. 26, 303–311.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.
Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R.C., 1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3, 294–299.
Gatesy, J., Baker, R.H., 2005. Hidden likelihood support in genomic data: can forty-five wrongs make a right? Syst. Biol. 54, 483–492.
George, W., 1981. Wallace and his line. In: Whitmore, T.C. (Ed.), Wallace's Line and Plate Tectonics. Clarendon Press, Oxford, pp. 3–8.
Giribet, G., 2005. Generating implied alignments under direct optimization using POY. Cladistics 21, 396–402.
Giribet, G., Boyer, S.L., 2002. A cladistic analysis of the cyphophthalmid genera (Opiliones, Cyphophthalmi). J. Arachnol. 30, 110–128.
Giribet, G., Carranza, S., Baguñà, J., Riutort, M., Ribera, C., 1996. First molecular evidence for the existence of a Tardigrada + Arthropoda clade. Mol. Biol. Evol. 13, 76–84.
Giribet, G., Edgecombe, G.D., 2006. Conflict between data sets and phylogeny of centipedes: an analysis based on seven genes and morphology. Proc. R. Soc. B 273, 531–538.
Giribet, G., Edgecombe, G.D., Wheeler, W.C., 2001. Arthropod phylogeny based on eight molecular loci and morphology. Nature 413, 157–161.
Giribet, G., Edgecombe, G.D., Wheeler, W.C., Babbitt, C., 2002. Phylogeny and systematic position of Opiliones: a combined analysis of chelicerate relationships using morphological and molecular data. Cladistics 18, 5–70.
Giribet, G., Kury, A.B., 2007. Phylogeny and biogeography. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, MA, pp. 62–87.
Giribet, G., Rambla, M., Carranza, S., Baguñà, J., Riutort, M., Ribera, C., 1999. Phylogeny of the arachnid order Opiliones (Arthropoda) inferred from a combined approach of complete 18S and partial 28S ribosomal DNA sequences and morphology. Mol. Phylogenet. Evol. 11, 296–307.
Giribet, G., Richter, S., Edgecombe, G.D., Wheeler, W.C., 2005. The position of crustaceans within the Arthropoda—evidence from nine molecular loci and morphology. In: Koenemann, S., Jenner, R.A. (Eds.), Crustacean Issues 16: Crustacea and Arthropod Relationships. Festschrift for Frederick R. Schram. Taylor & Francis, Boca Raton, pp. 307–352.
Giribet, G., Vogt, L., Pérez-González, A., Kury, A.B, submitted for publication. A multilocus approach to harvestman phylogeny with emphasis on biogeography and the phylogeny of Laniatores Cladistics.
Goloboff, G.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics 15, 415–428.
Goloboff, G.A., 2002. Techniques for analyzing large data sets. In: Desalle, R., Giribet, G., Wheeler, W. (Eds.), Techniques in Molecular Systematics and Evolution. Brikhäuser Verlag, Basel, pp. 70–79.
Hall, R., 2002. Cenozoic geological and plate tectonic evolution of SE Asia and the SW Pacific: computer-based reconstructions and animations. J. As. Earth Sci. 20, 353–434.
Hormiga, G., Arnedo, M., Gillespie, R.G., 2003. Speciation on a conveyor belt: sequential colonization of the Hawaiian Islands by *Orsonwelles* spiders (Araneae, Linyphiidae). Syst. Biol. 52, 70–88.
Kocher, T.D., Thoma, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc. Natl. Acad. Sci. USA 86, 6196–6200.
Kury, A.B., 1993. Análise filogenética de Gonyleptoidea (Arachnida, Opiliones, Laniatores). PhD Thesis. Universidade de São Paulo, São Paulo, 73pp.
Kury, A.B., 2003. Annotated catalogue of the Laniatores of the New World (Arachnida, Opiliones). Rev. Ibér. Arachnol., vol. especial monográfico 1, 1–137.
Kury, A.B., 2007. Laniatores: historical systematic synopsis. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, MA, pp. 159–177.
Linton, E.W., 2005. MacGDE: genetic data environment for MacOS X. Software. Available from: <http://www.msu.edu/lintone/macgde/>.
Loman, J.C.C., 1900. Über die geographische Verbreitung der Opilioniden. Zool. Jb., Abt. Syst., Ökol. u. Geog. Tiere 13, 71–104.
Lydekker, F.R.S., 1915. Wild Life of the World. Frederick Warne and Co., Ltd., New York.
Martens, J., 1976. Genitalmorphologie, System und Phylogenie der Weberknechte (Arachnida, Opiliones). Entomologica Germanica, Zeitschrift für das Gesamtgebiet der wissenschaftlichen Entomologie 3, 51–68.
Martens, J., 1980. Versuch eines Phylogenetischen Systems der Opiliones. In: Proceedings 8th International Congress of Arachnology, Vienna, pp. 355–360.
Martens, J., 1986. Die Grossgliederung der Opiliones und die Evolution der Ordnung (Arachnida). Actas X Congr. Int. Arachnol., Jaca 1, 289–310.
Martens, J., Hoheisel, U., Götze, M., 1981. Vergleichende Anatomie der Legeröhren der Opiliones als Beitrag zur Phylogenie der Ordnung (Arachnida). Zoologische Jahrbücher, Abteilung für Anatomie und Ontogenie der Tiere 105, 13–76.
Martens, J., Schwendinger, P.J., 1998. A taxonomic revision of the family Oncopodidae I. New genera and new species of *Gnomulus* Thorell (Opiliones: Laniatores). Rev. Suisse Zool. 105, 499–555.
Mayr, E., 1953. Fragments of a Papuan ornithogeography. In: Proceedings of the 7th Pacific Science Congress, New Zealand, vol. 4, 2 February to 4 March, 1949, Auckland and Christchurch, New Zealand, pp. 11–19.
Murienne, J., Harvey, M.S., Giribet, G., 2008. First molecular phylogeny of the major clades of Pseudoscorpiones (Arthropoda: Chelicerata). Mol. Phylogenet. Evol. 49, 170–184.
Myers, N., Mittermeler, R.A., Mittermeler, C.G., da Fonseca, G.A.B., Kent, J., 2000. Biodiversity hotspots for conservation priorities. Nature 403, 853–858.
Nixon, K.C., 1999. The Parsimony Ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.
Özdikmen, H., Kury, A.B., 2007. Replacement names for *Oncopus* and Oncopodidae (Arachnida, Opiliones). J. Arachnol. 35, 407–408.
Park, J.K., Ó Foighil, D., 2000. Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. Mol. Phylogenet. Evol. 14, 75–88.
Pérez González, A., 2006. Revisão sistemática e análise filogenética de Stygnommatidae (Arachnida: Opiliones: Laniatores). PhD Thesis. Museu Nacional. Universidade Federal de Rio de Janeiro, Rio de Janeiro, 308pp.
Pineau, P., Henry, M., Suspène, R., Marchio, A., Dettai, A., Debruyne, R., Petit, T., Lécu, A., Moisson, P., Dejean, A., Wain-Hobson, S., Vartanian, J.-P., 2005. A universal primer set for PCR amplification of nuclear histone H4 genes from all animal species. Mol. Biol. Evol. 22, 582–588.
Pinto-da-Rocha, R., 1997. Systematic review of the neotropical family Stygnidae (Opiliones, Laniatores, Gonyleptoidea). Arquivos de Zoologia 33, 163–342.
Pinto-da-Rocha, R., Kury, A.B., 2003. Phylogenetic analysis of *Santinezia* with description of five new species (Opiliones, Laniatores, Cranaidae). J. Arachnol. 31, 173–208.
Posada, D., 2005. Modeltest 3.7. Program and documentation. Available from: <http://darwin.uvigo.es/>.
Posada, D., Buckley, T., 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Syst. Biol. 53, 793–808.
Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818.
Prendini, L., Crowe, T.M., Wheeler, W.C., 2003. Systematics and biogeography of the family Scorpionidae (Chelicerata: Scorpiones), with a discussion on phylogenetic methods. Invertebr. Syst. 17, 185–259.
Prendini, L., Weygoldt, P., Wheeler, W.C., 2005. Systematics of the *Damon variegates* group of African whip spiders (Chelicerata: Amblypygi): evidence from behaviour, morphology and DNA. Org. Div. Evol. 5, 203–236.
Rokas, A., Williams, B.L., King, N., Carroll, S.B., 2003. Genome-scale approaches to resolving incongruence in molecular phylogenies. Nature 425, 798–804.
Roewer, C.F., 1923. Die Weberknechte der Erde. Systematische Bearbeitung der bisher bekannten Opiliones. Verlag von Gustav Fisher, Jena.
Ronquist, F., Huelsenbeck, J.P., 2005. Bayesian analysis of molecular evolution using MrBayes. In: Nielsen, R. (Ed.), Statistical Methods in Molecular Evolution. Springer, New York.
Schultz, J.W., 1998. Phylogeny of Opiliones (Arachnida): an assessment of the "Cyphopalpatores" concept. J. Arachnol. 26, 257–272.
Schultz, J.W., Pinto-da-Rocha, R., 2007. Morphology and functional anatomy. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, MA, pp. 21–33.
Shultz, J.W., Regier, J.C., 2001. Phylogenetic analysis of Phalangida (Arachnida, Opiliones) using two nuclear protein-encoding genes supports monophyly of Palpatores. J. Arachnol. 29, 189–200.
Schwendinger, P.J., 1992. New Oncopodidae (Opiliones: Laniatores) from southeast Asia. Rev. Suisse Zool. 99, 177–199.
Schwendinger, P.J., 2006. A taxonomic revision of the family Oncopodidae VI. Martensiellus, a new genus from Borneo, and the discovery of a tarsal pore organ in Oncopodidae (Opiliones: Laniatores) Zootaxa 1325, 255–266.
Schwendinger, P.J., 2007a. Oncopodidae. In: Pinto-da-Rocha, R., Machado, G., Giribet, G. (Eds.), Harvestmen: The Biology of Opiliones. Harvard University Press, Cambridge, MA, pp. 211–214.
Schwendinger, P.J., 2007b. A taxonomic revision of the family Oncopodidae VII. A new *Oncopus* species (Opiliones: Laniatores) from eastern Kalimantan. Rev. Suisse Zool. 114, 729–733.
Schwendinger, P.J., Giribet, G., 2005. The systematics of the south-east Asian genus *Fangensis* Rambla (Opiliones: Cyphophthalmi: Stylocellidae). Invertebr. Syst. 19, 297–323.
Schwendinger, P.J., Martens, J., 1999. A taxonomic revision of the family Oncopodidae II. The genus *Gnomulus* Thorell (Opiliones: Laniatores). Rev. Suisse Zool. 106, 945–982.
Schwendinger, P.J., Martens, J., 2002a. A taxonomic revision of the family Oncopodidae III. Further new species of *Gnomulus* Thorell (Opiliones: Laniatores). Rev. Suisse Zool. 109, 47–113.
Schwendinger, P.J., Martens, J., 2002b. Penis morphology in Oncopodidae (Opiliones: Laniatores): evolutionary trends and relationships. J. Arachnol. 30, 425–434.

Schwendinger, P.J., Martens, J., 2004. A taxonomic revision of the family Oncopodidae IV. The genus *Oncopus* Thorell (Opiliones: Laniatores). Rev. Suisse Zool. 111, 139–174.

Schwendinger, P.J., Martens, J., 2006. A taxonomic revision of the family Oncopodidae V. *Gnomulus* from Vietnam and China, with the description of five new species (Opiliones: Laniatores). Rev. Suisse Zool. 113, 595–615.

Sharma, P., Giribet, G., in press. A relic in New Caledonia: phylogenetic relationships of the family Troglosironidae (Opiliones: Cyphophthalmi). Cladistics 25.

Šilhavý, V., 1969. *Ibantila cubana* gen. nov. spec. nov., the first representative of subfamily Ibaloniinae Roewer (Arach., Opilionidea) from America. Acta ent. Bohemoslovaca 33, 372–376.

Smith, M.S., 1943. The biogeographic division of the Indo-Australian Archipelago, 3. The divisions as indicated by the Vertebrata. Proc. Linn. Soc. Lond. 154, 120–165.

Stamatakis, A., 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.

Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML web-servers. Syst. Biol. 75, 758–771.

Thorell, T.T.T., 1876. Sopra alcuni Opilioni (Phalangidea) d'Europa e dell'Asia occidentale, con un quadro dei generi europei di quest'ordine. Ann. Mus. Civ. Stor. Nat. Genova 8, 452–508.

Thorell, T.T.T., 1890. Aracnidi di Nias e di Sumatra raccolti nel 1886 dal Sig. E. Modigliani. Ann. Mus. Civ. Stor. Nat. Genova 10, 5–106.

Thorell, T.T.T., 1891. Opilioni nuovi o poco conosciuti dell'Arcipelago Malese. Ann. Mus. Civ. Stor. Nat. Genova 30, 669–770.

Varón, A., Vinh, L.S., Bomash, I., Wheeler, W.C., 2008. POY 4.0 Beta 2885. American Museum of Natural History. Software available from: <http://research.amnh.org/scicomp/projects/poy.php/>.

Wheeler, W.C., 1990. When is an outgroup not an outgroup and how to root DNA sequence based topologies without an outgroup. Cladistics 6, 363–367.

Wheeler, W.C., 1995. Sequence alignment, parameter sensitivity, and the phylogenetic analysis of molecular data. Syst. Biol. 44, 321–331.

Wheeler, W.C., 1996. Optimization alignment: the end of multiple sequence alignment in phylogenetics? Cladistics 12, 1–9.

Wheeler, W.C., 2003. Implied alignment: a synapomorphy-based multiple-sequence alignment method and its use in cladogram search. Cladistics 19, 261–268.

Whiting, M.F., Carpenter, J.M., Wheeler, Q.D., Wheeler, W.C., 1997. The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. Syst. Biol. 46, 1–68.

Xiong, B., Kocher, T.D., 1991. Comparison of mitochondrial DNA sequences of seven morphospecies of black flies (Diptera: Simuliidae). Genome 34, 306–311.